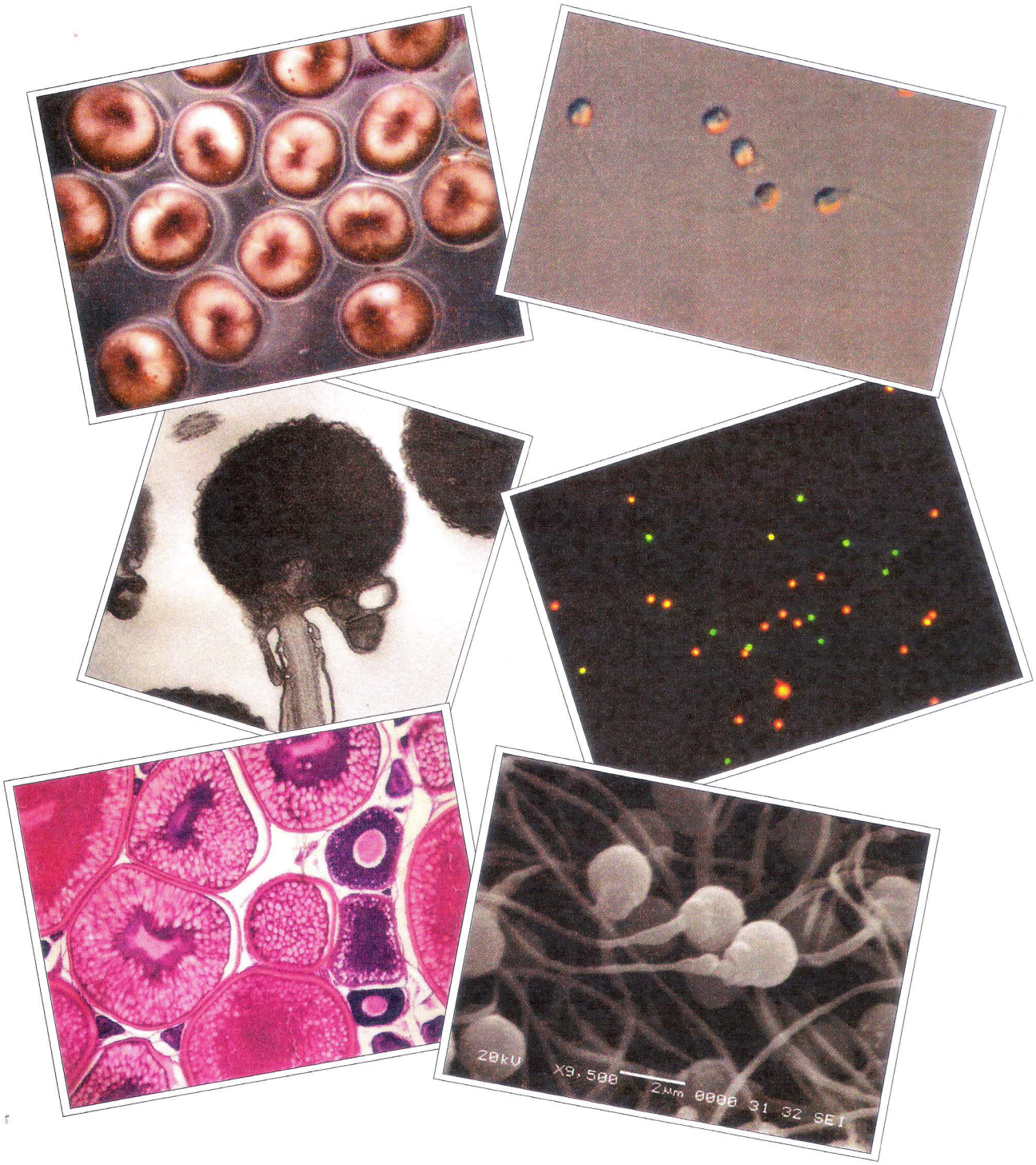
3RD INTERNATIONAL WORKSHOP ON THE BIOLOGY OF FISH GAMETES

SEPTEMBER 7-9, 2011

BUDAPEST, HUNGARY



FINAL PROGRAMME
BOOK OF ABSTRACTS

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SPERMATIC PARAMETERS OF *STEINDACHNERIDION PARAHYBAE* AMONG 10 AT 20 SECONDS POS-ACTIVATION USING OPEN SOURCE CASA

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Introduction

Fish sperm quality is often assessed by subjective methods, among which progressive sperm motility and sperm vigour are the ones most frequently observed. However, these measurements have raised doubts regarding their validation because the values obtained vary considerably, and basically depend on the experience of a single observer (Sanches et al., 2010). Lately, by means of specific software, fish sperm analyses have been conducted with computer programmes such as the *computer-assisted sperm analysis* (CASA) (Wilson-Leedy & Ingermann, 2007). However, for this method to be efficient is necessary basics knowledge about parameters utilized in the analysis such as, frame rate, number of frames, time of the video, and instant of evaluation. The present study was carried out with the aim of evaluate spermatic parameters of the method of CASA using open-source software among 10 the 20 seconds pos-activation in fish species threatened of extinction surubim-do-paraiba, *Steindachneridion parahybae*

Materials and methods

Thirteen *S. parahybae* males (639.6±104.2 g) which released semen under slight abdominal pressure were used. The semen collected (1.0 mL of each male) was used to capture video and further evaluation in software CASA. The semen was activated (distilled water) at a proportion of 1:50. Afterwards, 10 μL of the mixture were placed in a mirrored Neubauer chamber (100 μm deep), which was transferred to the light microscope for the capture of the videos (400x of the magnification). A Basler 602fc camera attached to a trinocular Nikon microscope was used. The videos were captured by the software AMCAP (Basler Vision Technologies) at a rate of 100 fps (656x490 pixels). The videos were captured in format *.avi, edited in the software VIRTUALDUB-1.9.0 (virtualdub.org), and exported as a sequence of images in format *.jpg. The images corresponding 0.5 second of video were opened, edited in the software IMAGEJ (National Institutes of Health, USA, http://rsb.info.nih.gov/ij/) and compiled using the application CASA (University of California and Howard Hughes Medical Institute, USA) according to Wilson-Leedy & Ingermann (2007) and Sanches et al. (2010). The analyses of sperm motility by application CASA were conducted after 10s of sperm activation, with 1s of interval until 20s pos-activation. For the semen originated from each male, the analyses were performed in triplicate. The sperm motility (MOT), curvilinear velocity (VCL), average path velocity (VAP) and straight line velocity (VSL) were submitted at ANOVA and linear regression analysis at 5% of significance.

Results

The spermatic parameters were different among time of 10 at 20s pos-activation ($P<0,05$), showed higher in 10s and lower in 20s, with inversely proportional relation ($P<0,05$) for all parameters evaluated (Figure 1). The number mean of spermatozoa evaluated was 61 for field visualization, this number is adequate for computed of fish sperm analysis (Wilson-Leedy & Ingermann, 2007). In the time of 10s after activation the values observed (means ± standard error) were 89.11 ± 1.25%, 107.23 ± 2.35 μm.s^{-1}, 83.58 ± 3.48 μm.s^{-1} and 77.08 ± 3.56 μm.s^{-1} for MOT, VCL, VAP and VSL respectively.

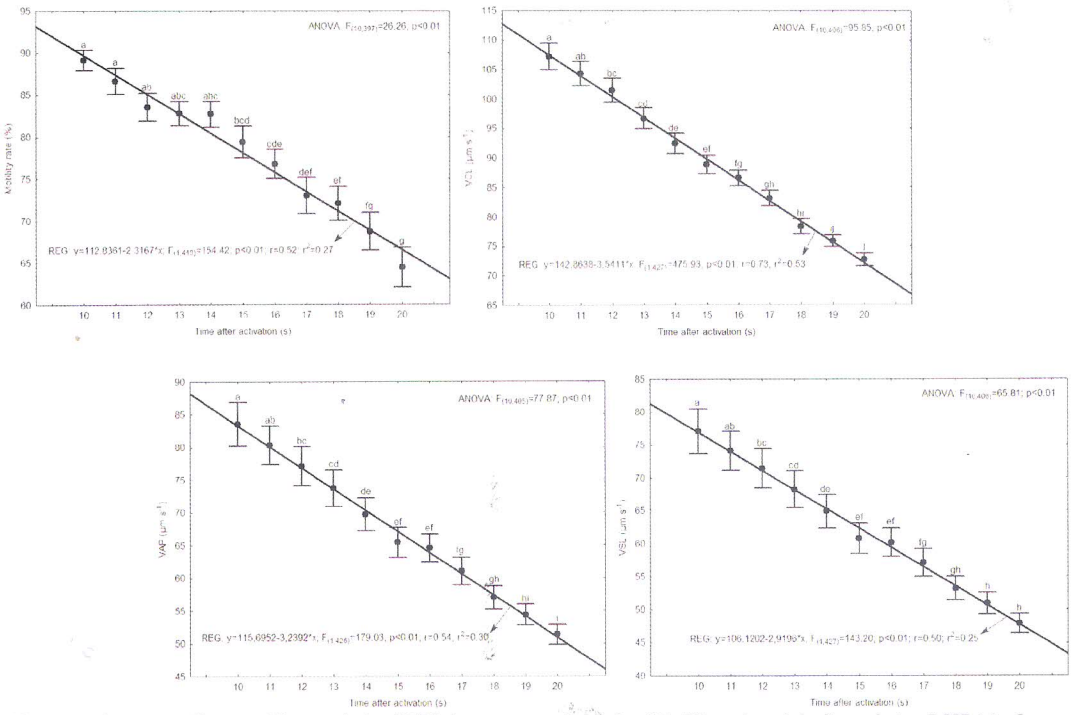

Figure 1. Sperm motility, curvilinear velocity (VCL), average path velocity (VAP) and straight line velocity (VSL) in S. parahybae among 10 at 20s pos-activation. Variance analysis (ANOVA). Linear regression analysis (REG). Different letters indicated (P<0,05) according Tukey test.

Discussion and conclusions

The behavior in *S. parahybae* sperms among 10 at 20s pos-activation (Figure 1) indicated continued reductions in sperm quality due to reduction of energy reserves (Cosson et al., 2010). These results are similar to those observed by Sanches et al. (2010) for a South American catfish, these authors observed reduction in sperm motility and velocity of *Rhamdia quelen* immediately after its activation. Given this we suggested that assessments of sperm parameters in *S. parahybae* immediately after activation, therefore this time may occur the highest rates of motility and velocity. However, lower times can be obtained with the experience of the person who is reviewing. Assessments for fish sperm in the short time that show activation (Billard & Cosson, 1992) is a limiting factor, especially when it comes to computer analysis that require standardization of several parameters (Sanches et al., 2010). The implementation of computerized sperm analysis presents an important tool for describing the behavior of sperm (Wilson-Leedy & Ingermann, 2007). The CASA open source software is rapid, exact and objective method for assessing to spermatic parameters of *S. parahybae*.

Acknowledgements

CNPq (number 478347/2009-0), FAPESP (number 2009/18609-6) and CESP.

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